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Agricultural Research

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Devastating Beauty
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Hard-Fought Victories Against Animal Diseases

The battles are not written up in history books or studied by legions of schoolchildren; monuments to the heroes are scarce. But the impact of the fight against animal disease echoes every day in the quality and quantity of foods available to consumers worldwide.

In March 1884, reports of foot-and-mouth disease in neighborhood cattle herds were enough to stir near hysteria in Coffey County, Kansas. Today, foot-and-mouth disease is only a memory in the United States, vanished from this country since 1929.

Foot-and-mouth disease is just one of at least a dozen major livestock diseases eradicated in the United States through the efforts of several USDA agencies, including the Agricultural Research Service which was created following the dissolution of the old Bureau of Animal Industry.

Perhaps ironically in light of the efforts that followed, when the U.S. Department of Agriculture was established in 1862, no provision was made for work on either animal husbandry or veterinary medicine.

It was not until May 29, 1884, that President Chester A. Arthur signed the act establishing the Bureau of Animal Industry within USDA. Feelings may have been ambivalent about the new bureau; while the act establishing it included an appropriation of \$150,000, the bureau at first was specifically limited to only 20 employees.

That rule eventually fell by the wayside, and the BAI evolved and expanded along with animal health concerns. Sweeping changes within USDA in 1953 saw the department's scientific bureaus, including BAI and the Bureau of Dairy Industry, abolished and their functions transferred to the then-new Agricultural Research Service.

ARS has been an important ally of the American farmer and consumer in combating animal disease. In the case of foot-and-mouth disease, which still threatens to re-enter the country from abroad, ARS' Plum Island Animal Disease Center in New York has played a part in the search for better protection against this dreaded attacker.

Researchers at Plum Island, working with scientists from the animal health industry, have developed technology that may someday lead to a more effective vaccine against foot-and-mouth disease.

Hog cholera, a bane to American farmers since its appearance in the Ohio Valley in the 1830's, today represents another animal health success story with a strong ARS connection.

Within a half century of its U.S. debut, hog cholera had spread to at least 35 states; a single Indiana company lost 11,000 hogs in the fall of 1896.

Scientists at ARS' National Animal Disease Center at Ames, Iowa, did their part to help drive out hog cholera by

refining new, rapid laboratory tests for diagnosis, in part so that the tests could be practically applied in a field program.

The tests, based on examination of tissue from animals' tonsils, were judged up to 90 percent effective. Additional refinements to these tests made it possible to pinpoint obscure outbreaks of infection that might otherwise have gone undetected.

Screwworms were a plague on the cattle industry of the South and Southwest until an ARS entomologist devised a new way to control the devastating pests.

The plan was to rear screwworms in large numbers, sterilize them, and release them in infested areas to mate with normal insects. Since no offspring would result from such matings, it was hoped that the pest would, in effect, breed itself out of existence.

The theory was tested on the island of Curacao off the coast of Venezuela; within 6 months, screwworms had vanished from the island. Subsequent application of the same technique on a massive scale led to the total eradication of this pest from the United States.

Poultry farmers have ARS researchers to thank for the vaccine now used against Marek's disease, at one time a major cause of deaths of birds and condemnation for tumors by USDA inspectors at slaughterhouses.

English researchers in 1967 reported the isolation of the causative agent of Marek's disease, a herpes virus. The causative agent was also discovered that same year at an East Lansing, Michigan, laboratory established by the BAI in the 1930's but now operated by ARS.

The East Lansing scientists found a herpes virus in turkeys that would not kill chickens but was closely related to the Marek's disease virus. From that, they developed a vaccine that today protects poultry around the world.

Producers are not the only ones to benefit from such research. The benefit-cost index of the Marek's disease vaccine to the production of human food was estimated in the mid-1980's at 44.3, meaning that a dollar spent on research would return an average of \$44.30 in economic benefits through such avenues as decreased costs of production of poultry meat and eggs.

It should also be noted that Marek's disease was the first cancer shown to be caused by a herpes virus and the first such condition of any animal to be controlled by a commercially applicable vaccine. A former director of the National Cancer Institute called the findings "one of the single most important developments in cancer research within the past 10 years."

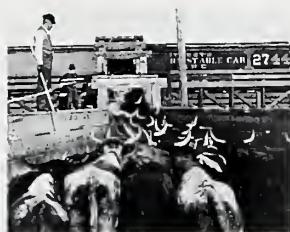
So despite whatever unspoken fears may have lurked when the Bureau of Animal Industry was originally limited to a staff of 20, that initial appropriation of \$150,000 has proven to be a wise and highly rewarding investment in the health and well-being of Americans both urban and rural.

Sandy Miller Hays
Information Staff
Agricultural Research Service

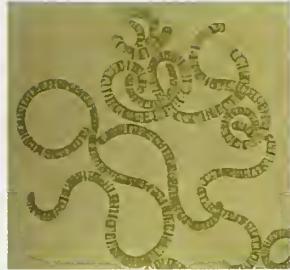
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Cover: Livestock sickness and deaths from eating poisonous plants such as lupine cost Western ranchers millions of dollars each year. Photo by Jack Dykinga. (K-4381-1)



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Vol. 39, No. 12
December 1991

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Agricultural Research is published monthly by the Agricultural Research Service, U.S. Department of Agriculture, Washington, DC 20250-2350.

The Secretary of Agriculture has determined that publication of this periodical is necessary in the transaction of public business required by law.

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Subscription requests should be placed with the Superintendent of Documents, Government Printing Office, Washington, DC 20402. Please see back cover for order form.

Address magazine inquiries or comments to: The Editor, Information Staff, Room 316, Bldg. 005,

10300 Baltimore Ave., Beltsville Agricultural Research Center-West, Beltsville, MD 20705-2350. Phone (301) 344-3280. After December 16, phone (301) 504-6280.

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Home on the Risky Range

Toxic Encounters With Range Plants

An only-one-of-its-kind-in-the-world lab in Utah works to make the range a safer place for grazing livestock.



On high mountain rangelands in central Utah, range technician Gus Warr collects larkspur samples to measure alkaloid levels. (K-4377-14)



In summer, purplish-blue larkspur blossoms decorate mountain hillsides and meadows in the western United States. But, beautiful as they are, these plants can be deadly when cattle feed on their foliage.

Larkspur, like pine needles, locoweed, lupine, and a host of other plants found in western pastures, contains toxins that can poison unwitting, hungry livestock.

When pregnant animals graze certain poisonous plants, the consequences can be especially gruesome—an offspring with twisted, deformed legs caused by toxins in lupine, or the bizarre, one-eyed lamb that can result when ewes eat false hellebore, a plant in the lily family.

Western ranchers suffer losses to the tune of \$340 million each year because of poisonous plants, according to Lynn F. James, research leader at the ARS Poisonous Plants Research Laboratory.

Located in Logan, Utah, about a 2-hour drive north of Salt Lake City, the laboratory is the only one of its kind in the world. James and his team of scientists work to identify toxic plants and their poisons, describe their effects, and develop new ways to prevent poisonings.

The loss figure related to poisonings includes only deaths and reproductive disturbances. But there are other, less

tangible costs, such as reduced weight gains in ill animals. Other indirect expenses include fencing pastures, herding cattle to steer them away from dangerous plants, and poisonous plant eradication efforts.

Hundreds of plants growing on western ranges can harm grazing livestock. Some have killed humans, too: the ancient Greeks executed the philosopher Socrates with a lethal tea brewed from hemlock. Today, people sometimes eat the bulbs of a grassy perennial called deathcamas, mistaking them for edible roots.

In terms of cattle losses, however, some of the worst offenders are larkspur, ponderosa pine, and locoweed.

U.S. ranchers in the intermountain West lose more cattle to larkspur than to any other poisonous plant. Cows like to eat the plants with spur-shaped flowers, which grow up to 6 feet tall in dense, abundant clumps in mountain pastures throughout the West.

Chemist Gary D. Manners, with the ARS Plant Protection Research Unit in Albany, California, has identified at least 15 different toxins, compounds known as alkaloids, in a single larkspur plant.

In cooperation with Logan animal scientist Kip E. Panter, he is trying to pinpoint which alkaloid is responsible for the plant's nasty effects on cattle.

JACK DYKINGA



A death from larkspur on mountain rangeland. (K-4382-2)

Once Manners identifies the toxin, he and Logan range scientist Michael H. Ralphs will determine whether the toxin's concentration in the plant changes in response to variables like temperature, moisture, or shade.

Trained Cows and Peer Pressure

With that information, they may be able to predict when the risk of poisoning is greatest. Logan scientists can then help ranchers work out the safest grazing strategies.

Tactics to stop larkspur poisonings take a variety of forms. One approach is simply killing the plants with herbicides. Ralphs, along with Utah State University scientists, has studied several promising chemicals, including metsulfuron, that work at very low concentrations on young larkspur.

"Herbicides may be useful to reduce dense patches of larkspur that cause poisonings year after year," says Ralphs. Widespread use, however, isn't practical because of the expense and because the plants often grow on rough, mountainous terrain that's not easily accessible.

If snuffing out the plants doesn't work, an alternative may be training cattle to avoid the attractive flowers.

Ralphs has tried a novel strategy known as aversive conditioning. He fed the cows small amounts of fresh larkspur and then dosed them—through a stomach pump—with lithium chloride, a chemical that nauseates them. The cattle associated the taste of larkspur with the illness and subsequently refused to eat it.

Initially, the experiment was successful. Animals staunchly avoided the plants in meadows. But the trained cows reverted to eating larkspur when they were placed in a field with untrained cohorts who were happily devouring the stuff.

Continued research will show if the aversion can be strengthened to overcome the influence of their peers.

If a cow does gobble a few mouthfuls of larkspur, is there any other possible way to thwart the toxin? Maybe, says animal scientist James A. Pfister of the Logan lab.

Pfister is investigating another potential strategy, a miniature pump that could be surgically implanted under a cow's skin. The pumps would release a protective compound—as yet unidentified—that would counteract the larkspur poison.

The pump is about the size of a man's little finger, large enough to slowly release drugs for about 28 days. Eventually, researchers might be able to fashion smaller pumps or pellets that could be implanted under

the skin, similar to the implants now used in cattle to slowly release growth hormones.

"Larkspur is dangerous to cattle for about 6 weeks during summer, so during that time the pumps could be used to protect cattle," says Pfister.

He's working with scientists at Colorado State University to identify a protective compound.

"Larkspur toxins seem to interfere with chemical compounds found at the nerve junctions. We're looking for something that could counteract that effect," says Pfister.

Like larkspur, ponderosa pines are also abundant in the West. If pregnant cows eat the long, soft needles of the ponderosa pine during the final trimester of pregnancy, they're likely to abort their calves.

JACK DYKINGA



Near Miles City, Montana, animal scientist Robert Short (right) and rancher Tim Donnelly discuss ways to prevent cattle from eating ponderosa pine needles, often the cause of abortion in cattle. (K-2973-10)



JACK DYKINGA

Since sheep are less affected by larkspur, they may be used to reduce the amount of it in mountain pastures before cattle are allowed to graze. In this study, range scientist Michael Ralphs records the sheep's plant preferences. (K-4378-10)

"Even if the cows don't abort, they'll often have premature, smaller calves that aren't as healthy as normal calves," says James. "Pine needles may cause losses totaling \$20 million each year."

Robert Short, an ARS physiologist based in Miles City, Montana, works with the Logan scientists in efforts to identify the physiological mechanisms responsible for abortions. Their earlier studies found

changes in levels of progesterone, a hormone important in pregnancy, in cows that ate pine needles.

Affected cows also have a tendency to retain their placentas instead of expelling them after the calf is born. Infections can result, and the cow may take longer than usual to rebreed.

Researchers aren't sure why cows eat the needles, which are chockfull

of bitter chemicals. Pfister found, however, that nonpregnant animals eat as many pine needles as their pregnant sisters.

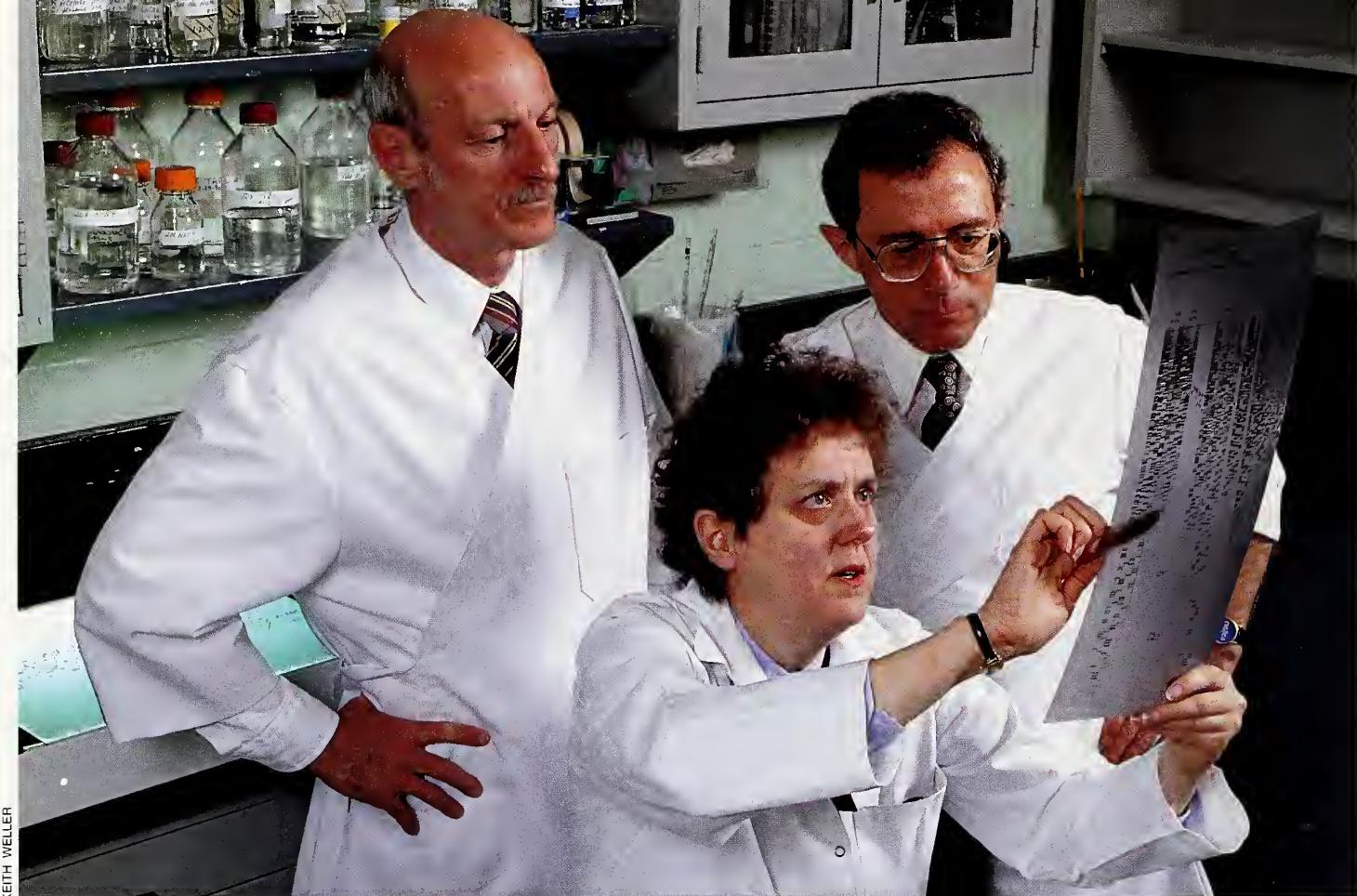
Other harmful plant toxins include alkaloid compounds found in lupines (members of the pea family), poison-hemlock, and various tobacco species, as well as larkspur. These can cause skeletal deformities such as cleft palate.

At the Logan lab, Kip Panter uses ultrasound imaging, common in human medical diagnosis, to observe developing animals whose mothers have eaten toxic plants. His aim is to learn exactly how and when the toxins harm fetuses.

"We've found that some of the alkaloid toxins actually sedate the growing fetus and keep it from kicking and stretching normally while it's in the uterus," says Panter. Consequently, calves' spines and legs may be crooked, instead of supple and flexible. In some herds, as many as 30 percent of calves suffer these deformities.

The ultrasound studies revealed that lupine and poison-hemlock are most dangerous when the pregnant cows graze them between the 30th and 70th day of gestation. Continued studies should narrow this "toxic window" even more.—By Julie Corliss, ARS.

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KEITH WELLER

Veterinary pathologist Norman Cheville (left), molecular biologist Shirley Halling, and National Animal Disease Center director Harley Moon analyze DNA sequence reactions of a vaccine made from a modified *Brucella abortus* bacterium. (K-4134-6)

Better Beefsteak Begins With Healthy Cattle

On the surface, it seems nothing could be simpler: Take a cow, add plenty of grass and water, and presto! Abundant protein-laden beef is the reward.

But that scenario doesn't reckon with the unseen hordes of bacteria and viruses that can thwart the best laid plans of an unwary cattle producer.

One of the most prevalent of these microscopic troublemakers is a bacterium called *Brucella abortus*, the culprit behind bovine brucellosis, a serious problem in American cattle herds since at least the 1840's.

Sometimes called Bang's disease in honor of the Danish veterinarian who first isolated the disease organism, brucellosis causes cows to abort, interferes with fertility, weakens calves, and lowers milk yields.

Each year, the disease costs U.S. cattle producers an estimated \$30

million, including animal losses and expenses for testing and vaccination.

Infected herds must be placed under quarantine. Additionally, regulations against interstate shipment within the United States and trade barriers imposed by foreign countries require U.S. cattle producers to certify that export cattle and embryos are brucellosis-free.

When a cow gives birth or aborts a calf, billions of *B. abortus* bacteria are shed in her body fluids or in the afterbirth. Other cows can become infected when they lick the afterbirth; newborn calves can be infected as fetuses or by drinking milk from an infected mother.

The disease typically gains entry to a healthy herd through the introduction of an infected heifer or calf. Animals can also become infected while mingling with other stock at fairs and livestock shows.

Current bacteriological tests may take as long as 2 weeks to identify the brucellosis organism. But with PCR, it only takes a day.

Precautions can be taken to impede invasion by this stealthy disease. Producers adding breeding stock to their herd must ascertain that the animals they buy are certified brucellosis-free. Calves are typically vaccinated between the ages of 5 and 9 months, a primary means of control.

But while commercial vaccines do protect cattle against brucellosis, there are significant problems with the currently used vaccine known as strain 19.

This vaccine uses a live strain of the bacterium, so the vaccine itself can cause abortion if given to a pregnant cow. The live bacterium also poses the threat of infection for humans handling the vaccine.

In addition, some cattle producers are reluctant to use the vaccine because of inability to distinguish in testing between animals that have been vaccinated and those that have been naturally infected.

"All of these factors together have dramatically slowed down eradication," says Norman F. Cheville, leader for brucellosis research at the National Animal Disease Center at Ames, Iowa.

"Vaccinated animals that react positively to blood tests for the disease may be classified as possibly diseased and consequently destroyed."

Fortunately, ARS researchers are gaining ground on a practical means of sorting out infected animals from vaccinated ones.

The scientists face two challenges. First, they must develop an effective vaccine with some sort of harmless mutation to help distinguish it from natural infection. Second, they must develop a diagnostic test capable of discerning that difference.

Addressing the first task, ARS molecular biologists Shirley M. Halling and Fred M. Tatum have genetically modified *B. abortus* vaccine strain 19 in hopes of deleting a specific protein immunogen. The protein immunogen causes the cow's

immune system to produce the antibodies that typically indicate in testing that the cow is infected.

Currently, the protein immunogen is present in both the vaccine and in the disease-causing organism. If a vaccine lacking that protein is developed and used, Halling says, tests for the protein in an animal's blood could reveal whether the animal has received the altered vaccine or has been infected with a disease-producing strain of *B. abortus*.

The researchers are determining the effectiveness of three mutant vaccines on 24 cows and 24 calves.

be easily identified by a blood test," says Cheville.

"Then we'll know positively if an animal has been vaccinated with the mutant vaccine or has been infected naturally by a virulent field strain. Such an accomplishment would be a major breakthrough toward the eradication of brucellosis."

In a related project directed by Halling, microbiologist Betsy J. Bricker has tackled the job of improving the diagnosis. She's discovered a small piece of genetic material—DNA—that's found in all *Brucella*, but not in other bacteria.

Bricker uses a technique called polymerase chain reaction (PCR) to track down the brucellosis organism. This technique makes many copies of targeted genetic material. When these copies are exposed to ultraviolet light, DNA of a particular size glows, unmasking any *B. abortus* lurking there.

Current bacteriological tests may take as long as 2 weeks to identify the organism. "But with PCR, the organism can be identified in 1 day," she says.

The use of PCR in *B. abortus* diagnosis also eliminates the need for using live bacterial cells. Diagnosticians must use particular caution now in performing bacteriological tests with live cells because humans can contract brucellosis. Symptoms include extremely high fever and chills, fatigue, and an aching similar to that of arthritis.

In trials on 30 randomly selected field isolates of *B. abortus*, the PCR method correctly identified all 30 samples.

Bricker plans to design a test to identify other species of brucella such as *B. melitensis*, which infects goats, and *B. suis*, which infects pigs. She also hopes to expand the technique to identify *B. abortus* vaccine strain 19 or one of the new mutant vaccines.

KEITH WELLER



Using a polymerase chain reaction assay, microbiologist Betsy Bricker identifies *Brucella abortus* and *Brucella ovis* in field samples. (K-4142-1)

Two of the mutant vaccines used in the trials were genetically engineered by Halling and Tatum; the third is a natural mutant strain developed by scientists at Virginia Polytechnic Institute and State University.

"We're looking for the one that provides the best immunity and that can

Just as pregnancy poses obstacles to brucellosis vaccination, it can also interfere with herd vaccinations against infectious bovine rhinotracheitis (IBR), a severe respiratory disease of cattle.

Caused by bovine herpesvirus 1 (BHV 1), IBR can be blocked by live-virus vaccines. But most of these vaccines cannot be given to pregnant cattle because of the danger of abortion. And in some herd management systems, cattle producers may not know when their cows are pregnant or have just been bred.

At the National Animal Disease Center, veterinarian Janice M. Miller and microbiologist Cecelia A. Whetstone are testing a genetically altered version of BHV 1 that might be used to develop a safer vaccine against IBR.

Cooperating researchers at the University of Pennsylvania altered the virus by removing a piece of its genetic material that causes production of the enzyme thymidine kinase (TK). The ARS researchers then gave the altered virus to six pregnant cows and none of the cows aborted.

In contrast, five out of six pregnant cows given the normal TK-positive virus aborted. The researchers will now study the TK-negative virus for its effectiveness as protection against IBR.

In the meantime, Miller recommends vaccinating young heifers against IBR at 4 to 10 months of age, before they reach breeding age.

At the Animal Diseases Research Unit at Pullman, Washington, microbiologist David T. Shen and colleagues

are also addressing the question of bovine herpesviruses.

In addition to studying BHV 1, the Pullman researchers are tackling bovine herpesvirus 4, which has been implicated as a cause of infertility and abortion in cattle.

Shen and co-workers have produced specially designed probes that could be used to diagnose these two viruses. One type of probe, called a monoclonal antibody, can detect the presence of antibodies made by the cow's body in response to the virus.

The other, a nucleic acid probe made from a fragment of the virus' genetic material, will detect the virus itself.

"Because these probes are very sensitive, we might be able to track down the virus in a variety of samples from cattle, such as blood, tears, or

KEITH WELLER



Veterinarian Janice Miller observes cultured cells that are infected with bovine rhinotracheitis virus. The virus is being grown for use as an experimental vaccine. (K-4140-9)

semen," says Shen. Improved diagnosis could help researchers prevent further spread of the potentially costly diseases, he adds.

Back at the National Animal Disease Center at Ames, close attention is also being paid to mastitis, an important and costly disease in dairy cattle and sheep as well as beef cattle. To complicate matters, the incidence of this bacterial disease is increased by stresses such as calving.

Veterinary medical officer Marcus E. Kehrli, Jr., and physiologist Judith R. Stabel have found in studies at Ames that cows are most susceptible to mastitis just before and after calving.

They hypothesized that a natural protein called G-CSF, made by various cells, could lend a helping hand to the cow's immune system in times of stress. G-CSF stimulates bone marrow to produce white blood cells that help fight off infections.

In tests, Kehrli gave daily injections of G-CSF to 10 dairy cows. The researchers noted that after these injections, the cows' number of white blood cells, called neutrophils, soared to 15 times more than normal.

"If we can increase the number and ability of white blood cells to protect against infections, we may reduce the need to use antibiotics," says Kehrli. "This means that costs to producers would be reduced."

Also figuring into the link between disease and stress is the breed of cattle involved, according to physiologist Michael T. Zavy.

Stress sets off a chain reaction in an animal. The hypothalamus in the animal's brain secretes a chemical called CRF that causes the animal's pituitary to release a hormone known as ACTH. This hormone in turn acts on the animal's adrenal gland, causing the production of substances called glucocorticoids.

KEITH WELLER



Microbiologist Cecelia Whetstone is working on a safer IBR vaccine than the one currently used, which is made from a bovine herpes virus that can cause abortions in pregnant cattle. (K-4136-1)

Pinpointing the level of glucocorticoids is important because it's believed a chronic overabundance of glucocorticoids can interfere with the animal's natural immune response, leaving it wide open to a variety of infections.

In tests at the ARS Forage and Livestock Research Laboratory at El Reno, Oklahoma, Zavy studied differences in stress reaction between *Bos taurus* cattle, typically the British breeds, and the *Bos indicus* breeds, typified by Brahman cattle.

Since many herds in the southern United States include a combination of Brahman and British blood, Zavy's study included 10 calves from Brahman fathers and Angus mothers, 12 from Brahman fathers and Hereford mothers, and 22 from Angus fathers and Hereford mothers.

Over a 45-day period, the calves were separated from their mothers,

weaned, and hauled on cattle trucks for 21 hours. Throughout the test period, 11 blood samples were taken from each calf to check levels of a specific glucocorticoid, cortisol. In addition, each animal received an equal dose of ACTH during initial handling, weaning, transportation, and recovery to stimulate cortisol release.

"By doing all this, we could compare the amount of cortisol secreted at different times," says Zavy. "If the animals all reacted to the stress in the same way, they should theoretically have equal amounts of cortisol."

Overall, the tests indicated weaning and transport were most stressful for both *Bos taurus* and *Bos indicus* cattle. But they also uncovered some differences in how the two species handled stress.

"On a day-to-day basis, *Bos indicus* animals had up to 50 percent higher levels of cortisol, as well as higher levels of norepinephrine and epinephrine, than *Bos taurus*," Zavy says.

"But when we gave the ACTH, the *Bos taurus* animals mobilized more cortisol than the *Bos indicus*. It wouldn't be unreasonable to say this difference could affect disease-resistance capabilities among the different breeds. But this work is just a starting point; there's still a long way to go."

While transport is admittedly stressful, stopovers at the sale barn can also take a toll, says Andy Cole, an animal scientist and research leader at the ARS Bovine Respiratory Disease Research Unit at Bushland, Texas.

"The typical feeder calf in the United States is weaned at 6 to 7 months of age and goes to the auction barn, where it's purchased by a buyer who's putting together an order for a feeder or stocker operation," explains Cole.

"On average, it takes 4 days for an order to be assembled. That's 4 days when calves from as many as 60

different farms are mingled together and stressed from being separated from their mothers."

The result: A bacterium called *Pasteurella haemolytica*, commonly found in the calf's nasal cavity, may take advantage of the animal's stressed condition and invade its lungs, causing pneumonia. In addition, other bacteria and viruses, probably passed on from the calf's new penmates, attack and infect.

Fortunately for farmers, 14 years of research at Bushland have uncovered secrets for easing the calf's journey down the marketing path.

"The typical order buyer will feed the calves low-quality hay at the sale barn," Cole says. "But our data indicates that if you give them a nutritionally balanced concentrate plus good quality hay, the number of animals that get sick will be reduced up to 20 percent, and death rates are cut an average of 30 percent."

Cole says a typical feed concentrate might include corn as its grain, along with a protein supplement and cotton-seed hulls or ground alfalfa, for an overall protein content of about 14 percent. Using a 50-percent-grain feed concentrate of this type costs about 30 cents per calf, he adds.

One common method of pre-conditioning the calf for the rigors to come has been weaning it about 30 days before sale and switching it to feed concentrate at the farm.

"But our data indicate that's not economical," says Cole. "However, if you give them limited amounts of feed concentrate in addition to the milk from their mothers the last 60 days on the farm, they get more nutrition in their rumen, they become familiar with the concentrate, and they're stressed less when they're taken from their mothers."

One of the chief culprits in calves' stress-related ills is bovine viral diarrhea, or BVD. This virus is

KEITH WELLER



Physiologist Judith Stabel draws blood to be analyzed for the effects of an earlier injection of a white-blood-cell stimulating natural protein, G-CSF. (K-4135-11)

receiving close scrutiny from microbiologist Hwei-Sing Kwang and veterinary medical officer E. Travis Littledike at the Roman L. Hruska U.S. Meat Animal Research Center (MARC) at Clay Center, Nebraska.

"The mortality rate on BVD is usually low, but it sets the cattle up for many other diseases," says Littledike. "Cattle producers vaccinate their herds against this disease, but there's a big controversy over which vaccines to use. We found evidence that the strain of BVD we have here at MARC wasn't controlled by the vaccine we'd been using for the last 8 years."

Complicating the picture are carrier animals who do not respond to the vaccines.

"When a calf is exposed to the virus while still in its mother's uterus between the 45th and 125th day of pregnancy, the calf's immune system isn't programmed yet," Littledike explains. "So when the immune system is finally programmed, it assumes that virus is a normal part of

the animal's body and does not make antibodies against the virus."

The carrier animal can spread the virus to its herd mates through coughing, saliva, feces, or any other body secretion, notes Kwang.

"If we can pull the carriers out of the herd before breeding season begins, that should prevent further BVD problems in the herd, unless more BVD-infected animals are brought into the herd," Littledike adds.

"But because a carrier's immune system doesn't usually produce antibodies against the virus, it's hard to identify them with tests that check for antibodies. The virus has to be isolated and identified in the blood to detect carriers."

In addition to costing as much as \$25 per test, current methods to detect infection in an animal can take up to 2 weeks for results. In contrast, Kwang has devised a system that offers an answer in 1 to 2 days.

Kwang's system, which involves producing recombinant proteins in

bacteria from a piece of DNA of a BVD strain, might also help clarify whether the antibodies in a calf's blood are the result of an earlier vaccination or actual infection.

One telltale sign is the presence of specific proteins produced by the viral RNA. Animals naturally tend to produce antibodies against three such proteins—p80, gp53, and gp48—in greater abundance than against other proteins from the virus.

Kwang and Littledike have found no evidence of p80 in blood samples from animals that have been vaccinated with a killed strain of BVD. However, p80 was detected in animals that had been naturally infected or had received a modified live BVD vaccine. All three methods of exposure resulted in antibodies to gp53 and gp48.

Down the road, the scientists hope to speed the diagnostic process to a few hours' time. Meanwhile, they say they may be able to use the viral proteins to pinpoint the elusive carriers in large herds.

"We could inject a rabbit with a recombinant protein like gp53 or gp48 and use the rabbit antibodies against that protein to test the blood sample from an infected carrier," says Kwang. "If the rabbit antibodies reacted to the gp53 or gp48 protein in the blood, it would mean the virus is present in the suspected carrier's blood."

Internal problems of a more tangible type—parasites—are engrossing microbiologist Louis C. Gasbarre and animal scientist Andrew C. Hammond. Hammond is research leader at the ARS Subtropical Agricultural Research Station at Brooksville, Florida, while Gasbarre works at ARS' Helminthic Diseases Laboratory at Beltsville, Maryland.

The two researchers are cooperating on studies of how possible genetic differences among cattle affect their natural ability to resist parasite infection.

One of their chief research tools is a unique herd of Angus cattle known as the Wye Herd and owned by the University of Maryland. Once held by a private owner on Maryland's Eastern Shore, this closed herd has been so carefully developed that its genetic makeup is very well defined.

From 1986 to 1988, the researchers monitored every calf born in the Wye herd for parasite infection. Although most calves' natural defenses against internal parasites began working by a certain age, some animals continued to have unusually high numbers of parasites.

"We estimate 15 percent of the calf population is responsible for 80 percent of parasite transmission," says Gasbarre. "About 50 percent of the calves we studied had under 100 parasite eggs per gram of feces, and about 80 percent had under 200 eggs per gram. But a few had well over 1,000 eggs per gram."

By studying the calves' "family tree," the researchers have found strong

indications that the genetics of the sire heavily influence the calf's chances of increased parasite infection.

"This trait is about one-third genetically controlled," Gasbarre says. One immediate suspect in the transmission of this trait is a specific complex of genes responsible for an animal's natural internal defenses against threats ranging from tumors to allergies.

The researchers have classified the Wye Angus by their combinations of these crucial genes, known as BoLA for bovine lymphocyte antigens.

Selective breedings done with the Angus at Brooksville and at the Wye Research and Education Center at the University of Maryland have demonstrated little correlation between the sire's BoLA and parasite infection levels of its offspring. However, those experiments have verified that the sire strongly influences parasite levels.

"We're absolutely convinced that there's a strong genetic effect involved in parasite resistance, but it's not strictly from the BoLA genes," Gasbarre says. "It's other genes from the sire."

Ultimately, the scientists hope to discover differences at the cellular level that control whether a beef animal is more likely to have heavy parasite infection.

"Once we know that difference, we can look for some sort of marker," says Gasbarre. "The marker may not be actually responsible for the trait, but it would be something you could check for in a calf. Then you might treat those animals more intensively, or even remove them from the herd." —By **Sandy Miller Hays, Linda Cooke, and Julie Corliss, ARS.**

For addresses and telephone numbers of ARS scientists mentioned in this article, please contact the Editor, Bldg. 005, BARC-West, 10300 Baltimore Ave., Beltsville, MD 20705. Phone (301) 344-3280. ♦



Microbiologist Hwei-Sing Kwang and summer intern Aida Boghossian examine recombinant proteins to detect antibodies to bovine viral diarrhea. (K-4383-18)

Cattle and Sheep Together: Partners in Grazing

Cattle ranchers and sheep producers—their disputes are the heart of Old West legends and more than a few Hollywood epics. Now research has confirmed the foolishness of their long-ago fights and fears.

"Cattle, sheep, and even goats complement one another on rangeland," says range animal scientist Dean M. Anderson, based at the ARS Range Management Research Unit at Las Cruces, New Mexico.

"Not only have we found the three species make better use of rangeland because they eat different plants. We also discovered that if sheep and goats learn at an early age to stay with cattle, the cattle's presence protects the smaller animals from coyote predation."

Sheep and cattle grazing together normally don't associate with each other. But if the two animal species are

introduced soon after the lambs are weaned, that behavior changes.

"Then, lambs form a close social bond and develop an impelling need to be close to cattle as they move about on the open range," says animal physiologist Clarence V. Hulet, recently retired from the Las Cruces lab.

The ARS scientists found that enduring social bonds were formed when 45- to 90-day-old lambs were penned with docile yearling heifers for 30 to 60 days. The two species then freely intermingled as they roamed pastures on the agency's 193,000-acre Jornada Experimental Range near Las Cruces.

Hulet and Anderson point out that coexistence is nothing new in the animal kingdom. Various animals have shared feed in the same area for centuries. For example, this type of grazing still exists today on the

natural grassland ecosystem of the Serengeti in Tanzania and Kenya in east Africa among wildlife such as gazelles and zebras.

The ARS behavior-oriented grazing research adds a new twist to studies begun in the 1920's by the Texas A&M University Research Station at Sonora, Texas. In the 1950's and 1960's, studies of cattle, sheep, goats—and later deer—showed all the species do well together on shared range.

The scientists at Las Cruces have confirmed the dietary differences of these livestock—that cattle eat mainly grasses, while sheep and goats prefer broadleaf plants such as forbs and leaves from some small shrubs.

The Jornada research team is now studying a shrub called tarbush, one of four woody plant species ranchers had previously wanted to eliminate completely from their ranges to make room for forage grasses.

TIM McCABE



Bonded sheep and beef cattle on the Jornada Experimental Range. Cloth tape encircling the animals' bodies shows their bonding groups for long-distance identification. (K-2173-15)

"Our current research is aimed at understanding why some plants are palatable to livestock while others are ignored," says Kris M. Havstad, research leader and range scientist.

He and fellow researchers found livestock would eat up to 99 percent of newly formed leaves on some tarpush plants, while leaving other tarpush plants untouched.

This variability suggests some element in the plant attracts or repels grazing animals. The researchers think plants have specific chemical signatures that are recognized by an animal and directly affect palatability. They have begun detailed analyses of tarpush plant components.

"If we can determine how to safely increase the use of previously unused woody species, including honey mesquite, creosotebush, and broom snakeweed, we could tap an unused range resource," says Havstad. "Livestock grazing can be a tool to restore biological diversity without harmful environmental effects.

"It may be possible to condition young animals to include these plants in their diets, in much the same way we condition sheep and cattle to graze together."

A similar study in Idaho's upper Snake River plain also points up the potential benefits of multispecies grazing.

There, sheep nibble on forbs like arrow leaf balsam root, which sports bright yellow flowers, and the purple-blossomed hawksbeard. Meanwhile, cattle on the same pastures feast on bunch grasses such as bluebunch wheatgrass and speargrass.

"Cattle alone tend to graze grasses," says John W. Walker, a range scientist with the ARS Range Sheep Production Efficiency Research Unit in Dubois, Idaho. "But turning out a combination of animals to graze together helps preserve the area's natural diversity. If you put out just cattle that tend to prefer grass, the grass gets grazed year

JOHN STUMBOS



Healthy vegetation along Big Grizzly Creek in the Plumas National Forest. (K-4385-6)

JOHN STUMBOS



Study site shows effect of heavy grazing on streamside meadows. (K-4385-12)

after year, and that hurts its ability to compete with the other plant species, reproduce, and maintain itself in the plant community."

"With multi-species grazing, you evenly defoliate the forage species in the area, and their ability to compete for water and nutrients is more nearly equal," he says.

Early results from field studies suggest that lambs especially benefit from such an arrangement, since they gain slightly more weight when grazing with cattle than when grazing with sheep alone. Researchers think that's because there is less competition for the lambs' preferred plant species.

In California's Sierra Nevada mountains, researchers have focused on harmoniously blending livestock with nature.

They are studying a meadow that adjoins Big Grizzly Creek in the Plumas National Forest on the California-Nevada border, not far from Reno and Lake Tahoe, the site of a 4-year

study. Here, Nebraska sedge, tufted hairgrass, and baltic rush cover the floors of high mountain meadows, providing sustenance for cattle that graze the meadows periodically throughout the summer.

It's an ideal setting to measure the cycling of water and nutrients in mountain meadows and streams and to see how cattle grazing influences these cycles.

Of special interest in this study is the riparian zone, the span of lush, green vegetation alongside streams and other waterways.

"Riparian zones are important for lots of reasons—water quality and quantity, forage production, and wildlife habitat," says Gregg M. Riegel, a range scientist with the ARS Landscape Ecology of Rangelands Research unit in Reno, Nevada.

The ARS project, which began in the spring of 1989, calls for extensive studies of the meadow's soil, water, and plants. The researchers are

looking at how these are affected when a meadow is lightly grazed, moderately grazed, or left ungrazed.

When cattle graze, they consume nutrients like nitrogen and phosphorus that are stored in the plants. The animals' bodies convert these nutrients to muscle tissue—the steak or hamburger that ends up in supermarkets.

The scientists are measuring soil nutrients, plant growth, and water table changes near the stream, in the middle of the meadow, and near the edge of the forest.

"We feel that a better understanding of how these ecosystems work will help in future resource management decisions," says range scientist Tony J. Svejcar, one of the project's initiators. Formerly at the Reno research station, he is now research leader at the ARS

Range and Meadow Forage Management Research Unit in Burns, Oregon.

The early data hasn't shown any striking differences among any of the different grazing treatments. But the scientists have analyzed only a year's data thus far.

"One interesting finding was that the meadow contained a clay mineral called smectite," says Robert R. Blank, a soil scientist with the Reno lab.

"The mountain meadow isn't a typical environment for smectite to form in. A more arid setting would be more usual—an environment with a strong seasonal dry period and a more alkaline soil."

Because it sloughs easily, smectite may be more sensitive to the hooves of trampling cattle near the stream, he notes.—By **Dennis Senft and Julie Corliss, ARS**.

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JOHN STUMBOS



Range scientist Gregg Riegel checks voltage of electric fence that keeps cattle in specific research plots. (K-4384-6)

Animal Care Tightly Regulated

When it comes to protecting animals used in research, be they rats or cattle, the Agricultural Research Service goes the federal guidelines one better.

The Animal Welfare Act spells out rules for care and use of many types of animals in research. However, those rules do not cover laboratory rats and mice or agricultural animals used for production research.

But in ARS, "we cover all vertebrate animals, including fish," emphasizes Helene N. Guttman, Animal Care Coordinator for the ARS National Program Staff. "And that's not just ARS-owned animals, but also other animals used on ARS property, as well as animals in studies funded by ARS but done by ARS cooperators at their facilities."

Under ARS regulations, an Institutional Animal Care and Use Committee (IACUC) of at least five members must approve each individual experiment that uses animals at any ARS location, regardless of the source of funds for that research.

In addition, IACUC approval is required at non-ARS locations using ARS animals or ARS personnel, again regardless of the funding source.

This approval must be obtained before the experiment is begun—a process known as "protocol review."

Protocols are approved for no more than 12 months, so any experiment that continues for more than a year must be reviewed again at least annually.

The IACUC must include at least one doctor of veterinary medicine with training or experience in the species to be used in the experiment and one scientist who has experience in and is currently active in research involving animals.

Also required for the committee are one nonscientist, such as from businesses or the clergy, and one person who is not affiliated with ARS in any way other than as a member of the committee.

At these protocol reviews, the proposed experiments must survive close scrutiny, says Guttman.

"We ask questions such as, is this the appropriate species to use for this experiment? Is this an appropriate number of animals?" she explains.

"We also ask whether there are any verified nonintact animal methods,

Problems must be corrected quickly, and if the problem affects animal health, the animal is removed from the non-conforming facility.

such as tissue culture or computer modeling, that would give the needed answers to the same scientific questions."

Other key considerations include the training of the people who will perform the experiments and whether the best procedures have been selected to minimize pain or distress to the research animal.

In addition to the protocol reviews, the IACUC inspects all animal facilities at least once every 6 months. Included in these inspections are facilities belonging to ARS, those used by tenant agencies on ARS property, satellite facilities, and any indoor study areas where animals are held for more than 12 hours.

These inspections help ensure that no substantial change has been made in the experiment's approved procedures without IACUC approval of an amended protocol.

The IACUC prepares annual reports of animal use, signed by the IACUC chairperson and the veterinarian member. These reports go to the highest-placed person on the research location, to Guttman as ARS Animal Care Coordinator, to the director of the ARS administrative area where the research is being conducted, and, in the case of animals covered by the federal Animal Welfare Act, to other appropriate USDA authorities.

The IACUC also promptly investigates any complaints of animal abuse, nonconformance with an approved protocol, or noncompliance with ARS animal care and use rules. Problems must be corrected quickly, and if the problem affects animal health, the animal is removed from the nonconforming facility, Guttman says.

"In our animal research, we emphasize the '3 R's,' she adds. "Refine procedures to minimize animal pain or distress, reduce the number of animals to the minimum required for the experiment to be statistically meaningful, and whenever possible, replace animals with those lower in the evolutionary scale or with proven procedures that do not require the use of a live animal."—By **Sandy Miller Hays**, ARS.

Helene N. Guttman is the Animal Care Coordinator for the USDA-ARS National Program Staff, Room 105, Bldg. 002, BARC-W, 10300 Baltimore Ave., Beltsville, MD 20705-2350. Phone (301) 344-3423. ♦

Cattle Drives

Hard Times in the Old West

Sickening headaches, blisters and boils, lost mail, lost food, lost patience, and a multitude of vexatious flies: no, this wasn't a camping trip gone awry, but that most romantic of cowboy adventures, the cattle drive.

As many a starry-eyed young cowpoke soon discovered, there was little romance to the real thing—although perhaps more than in today's rumbling ride of a few hours up interstate highways in a tractor-trailer rig.

For starters, the old method wasn't really so much a cattle drive as a cattle walk, the idea being to move the animals to the far end of the trail without running too much "condition" off them. So as the working hours from dawn to dusk crawled by, a herd might step off only 12 to 15 miles a day.

Still, the era of the cattle drive was a sort of race against time. As the railroads spun their web across the West, the need to march cattle to distant railheads evaporated. In all, cattle drives boomed for only about 20 years, from the end of the Civil War to the mid-1880's.

For most ranches, the cattle drive was an annual affair. Frequently beginning in April or May, when grass and water were reasonably plentiful, some 500 to 2,000 cattle of all ages would be rounded up from around the ranch and sent trudging toward locales such as Sedalia, Missouri, or, after 1867, Abilene, Kansas.

The railhead towns, with their droves of cattle buyers waiting to deal, weren't always the destination. Some animals, particularly along the Good-night-Loving Trail [see map], were sold to government agencies for

allocation to Indians. Others were “seed stock” destined for secondary ranches in Montana and Wyoming.

The men nudging the herd on its way were not necessarily workers from the ranch that owned the stock. Often, professional trail hands escorted the herd.

The ratio of men to animals varied widely, sometimes stretching to just one man per 400 head. But the team's composition was fairly constant. Aside from the trail boss, who rode ahead of the herd to scout out fresh grass and water, there were point men to lead the herd; swing riders alongside the herd toward the front; flank riders keeping an eye on the sides toward the back; and a few poor souls riding drag, keeping tabs on stragglers and eating a nauseating amount of dust in the process.

Rounding out the team were the wrangler, in charge of the horse herd, or remuda; the cook with his chuck-wagon; and perhaps another driver and wagon hauling supplies or tools.

Another important team member was the lead steer, which walked at the head of the herd, encouraging the

A black and white historical photograph capturing a cattle drive. In the foreground, a cowboy wearing a wide-brimmed hat and a light-colored shirt is mounted on a dark horse, skillfully herding a massive herd of cattle across a dry, grassy landscape. The herd stretches across the frame, with cattle of various colors, mostly dark. In the background, another cowboy is visible on a horse, also engaged in herding. The scene is framed by a white border, and a text box in the upper left corner contains a letter from W. A. Carter advertising cattle for sale. The text in the box reads:

now bearing excellent prices East, it would be well
for persons to bring their Cattle forward as soon as
possible.
For further particulars, address
W. A. CARTER,
Fort Bridger, Wyo. Ter.
Fort Bridger, July 2, 1877.

CATTLE MEN READ THIS!
Great Inducements to those who wish to
Ship Cattle on the U. P. Railroad !!

Having entered into special arrangements with the U. P. R. R. Company, by which I can ship Cattle

the C. P. R. R. Company, by which train ship carts East at greatly reduced rates, and having selected a point between Carter and Church Buttes Stations

Having entered into special arrangements with the U. P. R. R. Company, by which I can ship Cattle East at greatly reduced rates, and having selected a point between Carter and Church Buttes Stations some ten miles East of the former place, near the junction of the Big and Little Muddies, and having Constructed Commodious Lots and Extensive Enclosures, and the Company having put in a Switch capable of holding 40 Cars, I will be Prepared to Commence Shipping on or before the 15th of the Present Month, and will be able to promptly ship any Number of cattle that may be Offered.

The cattle yards are in an enclosure of some 400 acres, and stock selected and confinement for breeding will be furnished. It appears that with cattle themselves will purchase, at good prices, all shipping cattle that may be offered. As cattle are now bearing excellent prices East, it would be well for persons to bring their Cattle forward as soon as possible.

For further particulars address

iculars, address
W. A. CARTER.

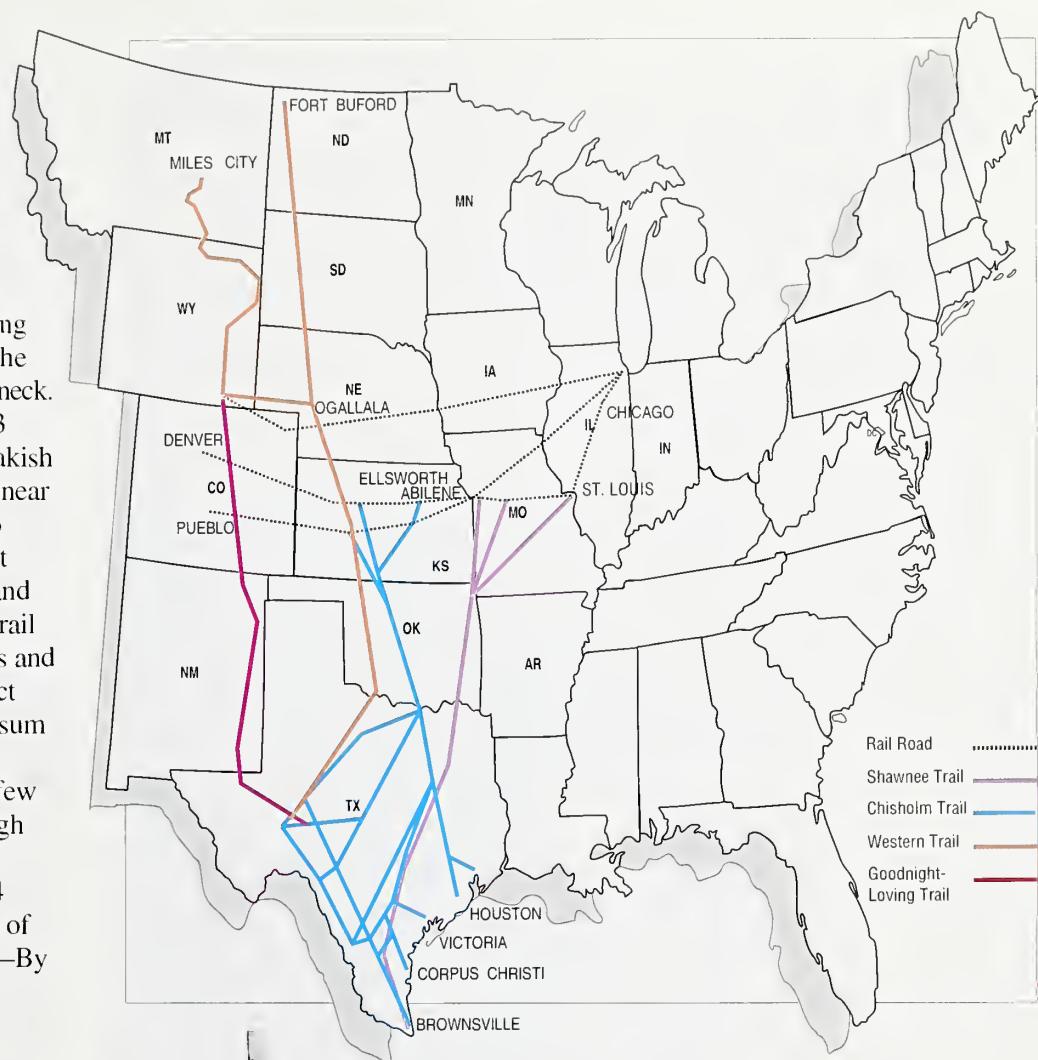
Fort Bragg

Fort Bridger, July 2, 1877.

others to follow. While many lead steers found only the slaughterhouse waiting at the end of the line, a few were recruited for repeat journeys. One such creature was Texas cattle king Charles Goodnight's Old Blue, which made the trip to Dodge City, Kansas, 8 times, luring some 10,000 head up the trail to the tune of the bell he wore around his neck.

If a cowboy survived the 2 to 3 months of sudden hailstorms, freakish lightning strikes, stampedes (one near Texas' Brazos River in July 1876 claimed 2,000 animals), thirst that could turn cattle blind and wild, and the gnawing knowledge that the trail boss' main concern was the cows and not the cowboys—he could collect \$100 in wages, hardly a princely sum even in the late 1800's.

There must have been quite a few takers, though. From 1867 through 1881, trail bosses found enough willing hands to herd more than 4 million beef cattle up the trail out of Texas to the northern railheads.—By Sandy Miller Hays, ARS. ♦



Facing page: Cattle roundup near Gallatin Valley, Montana in 1941. USDA photo.

At left: Loading cattle cars near Dickinson, North Dakota about 1898. Photo contributed by the State Historical Society of North Dakota

Infants To Benefit From Golden Eggs

In a deep freeze at the Children's Nutrition Research Center in Houston, Texas, lie the remains of the hen that laid "golden" eggs. Her 23 eggs—also in the deep freeze—cost \$1,300 each to produce. This is no fairy tale; it's real science. And the findings from this small study will be priceless to future research everywhere.

Nearly 99 percent of the carbon on Earth has an atomic weight of 12—except in this hen and her eggs. They have about 65 percent of the heavier nonradioactive isotope—13C. That's because this hen's diet was a lot more than chicken feed. It contained \$30,000 worth of an alga in which virtually all the carbon was 13C.

Researchers in the Center's Stable Isotope Laboratory are testing the feasibility of using the alga, *Spirulina*, to determine which amino acids are essential for infants—those that need to be gotten from the diet because the body doesn't make them—and under what conditions a nonessential amino acid may need to be in the diet.

If you think science already knows this, consider the findings with this hen. During her last 4 weeks, she got regular feed plus the 13C-labeled alga, which contains all the amino acids. Chicken experts have long believed that the amino acid proline is nonessential—that it can be made by chicks. But most of the proline in the hen's tissues contained all 13C atoms. The same was true for the proline she put in the egg whites.

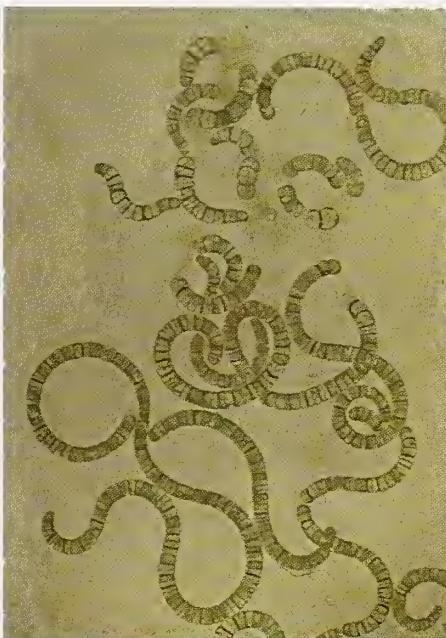
That meant the proline had come straight from the feed rather than undergoing breakdown and reassembly in the hen's liver, says Heiner K. Berthold, a visiting scientist supported by the German Research Society.

"This strongly implies that proline is an essential amino acid for the hen—at least under our experimental conditions." Berthold spent the last 2 years in Houston studying the priceless

chicken and her eggs. [His findings were published last September in the *Proceedings of the National Academy of Science*.]

Not only is this method much more sensitive than standard methods for determining the essentiality of amino acids, he says, "you can measure all 18 amino acids in one study." So far, Berthold and colleagues have developed the analyses for measuring at least 14 of them.

MARTEK CORPORATION



Spirulina alga grown in a high carbon 13 environment.

The classic method of determining essentiality is to leave a single amino acid out of the diet, he says. "You may trigger effects not directly related to that nutrient, or you may not see effects for weeks."

Obviously, such a study is not appropriate on infants, and there's still some debate as to whether infants can make enough of their own proline, arginine, and glycine when these amino acids aren't supplied in their diet.

"We know certain amino acids are essential. And there's a group that is conditionally essential: they are not made under certain circumstances. But we don't know the circumstances exactly," explains Berthold, a physician and clinical pharmacologist. "This method could be used to determine exactly which amino acids need to be supplied by the diet during periods of development and growth or in relation to the infant's calorie intake." Infant formula makers could then fine-tune their products to the precise needs of infants during the first few months of life.

Before they attempted to use the method on infants, Berthold and colleagues tested it on adults. Four women took a dose of the uniformly labeled alga, then ate regular foods throughout the day to simulate normal eating. In a second experiment, they fasted for 36 hours while consuming the alga to see if their livers had kicked in to produce the amino acids they didn't get from foods. Sure enough, they synthesized a larger proportion of several amino acids during fasting than they did during a day of normal eating.

The researchers focused on a protein found in very-low-density lipoprotein (VLDL)—the tiny globule that carries the bulk of fat circulating in the blood. Since this protein, B-100, has a short half-life—only a couple of hours—researchers can watch it come and go in under 8 hours.

Such a study can do far more than prove whether or not an infant can make an amino acid. It can show the degree and rate of absorption of amino acids as well as their metabolism.

For instance, after one subject consumed the dose of labeled alga, the 13C-labeled amino acids started appearing in blood plasma within a few minutes, peaking 1 hour later. And they began to be incorporated in the B-

100 protein within 40 minutes, peaking in 3 hours, says Berthold.

Using a uniformly labeled food source is like attaching a microscopic video camera to each amino acid and following its travels through the body. What's more, since all compounds in the alga are built on a backbone of ¹³C, researchers can study the fate of fatty acids, sugars, nucleic acids that make up DNA, and any other carbon-containing compounds.

A meal of *Spirulina* may not be considered haute cuisine, but it's a major source of protein in some parts of the world, says Berthold. Farmers in Africa and Mexico grow the alga in ponds, then dry out the ponds to harvest it. "It has an amino acid profile very similar to egg white," he notes.

The labeled *Spirulina*, however, was grown in a closed system by Martek, a company in Columbia, Maryland, specializing in algal products for research applications. All the carbon dioxide piped into the system contains the heavier ¹³C, says Peter Klein, who initiated the research with *Spirulina* and heads the Stable Isotope Laboratory. As a result, the proteins, fats, carbohydrates, and other organic compounds are uniformly labeled with ¹³C. Well...almost uniformly. About 3 percent of the carbon is the ubiquitous ¹²C, which slipped into the system unnoticed.

As for the hen and her 23 golden eggs, they won't go to waste. They can be used just like the alga in future studies. But for now, they've proved that a uniformly labeled food source can be a powerful scientific tool.—By Judy McBride, ARS.

Heiner K. Berthold and Peter D. Klein are at the USDA-ARS Children's Nutrition Research Center, 1100 Bates Street, Houston, TX 77030. Phone (713) 798-7000. ♦

DAVID NANCE



Physician and clinical pharmacologist Heiner Berthold uses the carbon 13 method to study amino acids at ARS' Children's Nutrition Research Center. (K-4397-1)

The future looks bleak for worms that ravage cotton. But it looks bright for growers weary of spending \$70 million or more a year on caterpillar insecticide. In a few years, they could get wormproof cotton varieties with a gene-engineered punch borrowed from bacteria.

Scientists at the Agricultural Research Service have just gathered the second harvest from cotton plants with the new genes. They're exploring ways to ensure the plants will be no flash in the pan.

Scientists at Monsanto Co. in St. Louis inserted the new genes, originally from the bacterium *Bacillus thuringiensis*, or Bt. It has been sold for 30 years as a natural control for caterpillars in crop fields, gardens, and forests. Bt makes a protein that starves a pest by paralyzing its digestive tract.

Similar proteins are made by the new plants, called transgenic since they hold genes transferred from a different type of organism. The plants all but wiped out tobacco budworms, cotton bollworms, pink bollworms, and other caterpillars in outdoor tests at seven sites in 1990 and six sites in 1991. In 1990, damage to Bt cotton plants was sometimes less than 1 percent, less than regular plants sprayed with chemicals. This year was a replay, say David Altman, Johnie Jenkins, and Doug Wilson of ARS.

In Arizona, "pink bollworm larvae started to

DAVID NANCE



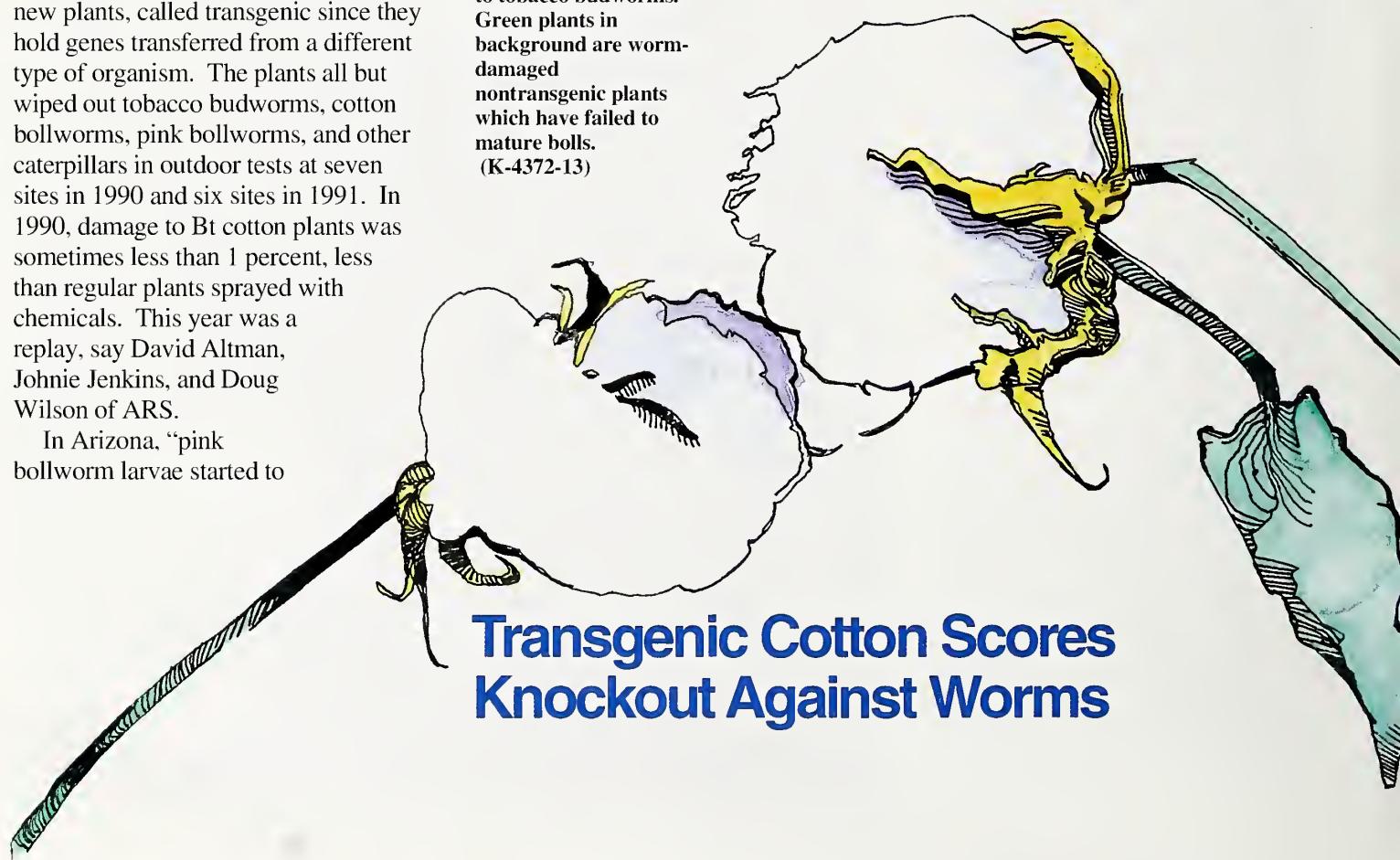
Geneticist Johnie Jenkins inspects healthy cotton bolls in a plot of Bt transgenic plants that were intentionally exposed to tobacco budworms.

Green plants in background are worm-damaged nontransgenic plants which have failed to mature bolls.
(K-4372-13)

feed on flowers but quit and died when they were still so small you could barely see them," says Wilson. He heads ARS Cotton and Insect Genetics Research at the Western Cotton Research Laboratory in Phoenix.

ARS tested the plants under a cooperative agreement with Monsanto. "The company came to ARS because they wanted an unbiased referee and we have three decades of experience studying insect resistance in cotton plants," says Jenkins, director of the Crop Science Research Laboratory, Starkville, Mississippi.

The agency also has expertise in Bt. Genes in this year's transgenic plants originated in HD-1 and HD-73 strains of the bacterium. HD stands for Howard Dulmage, who identified and tested many Bt strains during his ARS career.



Transgenic Cotton Scores Knockout Against Worms



Monsanto's Randy Deaton (left) and ARS geneticist Doug Wilson examine an unprotected cotton plant that has been heavily damaged by beet armyworms. (K-4374-1)

In 1991, more than 120,000 Bt plants from four transgenic lines were tested: 1990's two top performers—with HD-1 genes—and two lines with HD-73 genes tested at all the sites for the first time.

All the transgenics derive from a commercial cotton, Coker 312. Also planted were ordinary Coker 312 and a second, locally adapted nontransgenic variety. To aid comparisons, scientists sprayed insecticide on half of the plots.

ARS ran tests in Mississippi, Texas, and Arizona in cooperation with Mississippi State University, Texas

A&M University, and the University of Arizona. Other trials in Alabama, Texas, and Louisiana were run by Monsanto, Texas A&M, and Louisiana State University.

ARS scientists varied the transgenic challenge: in Mississippi, massive artificial infestations of tobacco budworms; in Texas, natural invasions of cotton bollworms as well as tobacco budworms; in Arizona, releases of thousands of pink bollworm moths plus raids from other leaf-chomping insects.

Overall, while the insect victims' names differed, they shared the same fate.

During July in Mississippi, Jenkins and ARS colleagues William L. Parrott and Jack C. McCarty found that tobacco budworms damaged as many as half the flower buds, or squares, on tattered Coker 312 plants unprotected by insecticide. Transgenic plants had 0 to less than 7 percent damaged buds.

In Texas, "Bt plants gave almost complete control of cotton bollworm and tobacco budworm. In unsprayed plants that didn't have a Bt gene, insects took over," says Altman, research geneticist at the Southern Crops Research Laboratory, College Station, Texas.

The same trend doomed pink bollworms at the 3-acre test site at Maricopa, Arizona. There, Doug Wilson and colleague Hollis Flint released 1,000 pink bollworm moths 3 times a week from mid-June to the end of July.

After mating early in the season, females lay eggs on various parts of the plant. When an egg hatches, the larva crawls to a bud, bores in, eats pollen, and spins a sticky web. The web keeps the flower from blooming properly. Such rosetted flowers incubate the first of as many as five generations of pink bollworms during the season. To see how successful that



critical first generation was, Flint checked the percentage of rosetted flowers.

In June and July, 2.3 percent of the flowers were rosetted on ordinary Coker 312. On transgenics, less than one in a thousand flowers were rosetted.

"Waylaying that first generation means much smaller numbers later," says Wilson.

"In mid-July we clipped samples of green bolls and incubated them to see how many larvae would emerge. Two weeks later we found 1 larva in 373 transgenic bolls. The Coker 312 had larvae in almost one-fourth of the bolls. In Arizona and California, that's about double the level at which the grower will get significant damage unless something's done—and right now that something is insecticide."

Wilson says the Bt protein also halted other lepidopteran insects such as cotton leafperforators, saltmarsh caterpillars, and beet armyworms.

Will Pests Keep Turning Belly Up to Bt?

The scientists all recognize that "while we had almost no damage on the Bt plants, they're not a cure-all," says Jenkins.

"Suppose that out of a million tobacco budworms, one male and one female have genetic resistance that keeps them healthy despite the Bt protein. If those two insects find each other and mate, you could soon have thousands of Bt-resistant caterpillars."

But that won't happen if the transgenic farming strategy stays focused on the real purpose of pest control.

"The real goal," says Jenkins, "is to kill enough insects, year to year, to keep damage below a level that would hurt the farmer's pocketbook."

Part of the strategy for foiling resistance is making sure a farmer's transgenic plants will reliably express the Bt protein. That's why cooperative studies by ARS, Mon-

JACK DYKINGA



Entomologist Hollis Flint compares an insect ravaged cotton leaf from a control variety with one that has been genetically engineered with a protective gene from *Bacillus thuringiensis*. (K-4376-11)

santo, and university scientists are aimed at supplying information for breeding dependable—as well as powerful—Bt genes into high-quality commercial cultivars.

In College Station, studies by Altman and Monsanto scientist Eric Sachs include thousands of assays of transgenic plant tissue—to learn how well the Bt genes are inherited and how powerfully they express the protein.

"The Bt protein is from a foreign gene," Altman says. "We can't assume it will behave like one that's been part of the cotton chromosome for thousands of years. Furthermore, experience suggests Bt genes will need help from other genes. Otherwise, insects could develop resistance just as they can in response to insecticides."

In the fall, he sent Monsanto seed from more than 100 painstakingly selected specimens of brand-new transgenic crossbreeds grown outdoors for the first time. At a Monsanto nursery in Hawaii, the seed will be planted to obtain seed supplies for outdoor tests against caterpillars next summer.

The seed came from crosses that Sachs and Altman made between Bt plants and more than 60 different lines of cotton with strong natural pest resistance. Some lines came from an Australian scientist; most originated in ARS' cotton repository in College Station.

"ARS is a leader in this kind of high-risk breeding research," says Altman. "It generally isn't done by industry or universities, with a few exceptions like Texas A&M and Mississippi State University."

The ARS and Australian cottons have high levels of terpenoid compounds toxic to pests; no nectaries—sap-yielding glands that occur in leaves, bracts, and flowers and supply energy for moths; or smooth leaves and stems lacking minute hairs that help insect eggs stick to a plant.

"If the smoothness trait, for example, halves the number of eggs on the plant, a Bt protein will need to work on a far smaller number of larvae," says Altman.

Could Bt fiber be a worrisome wrinkle? Textile mills may sym-

thize with a farmer's caterpillar woes, but they pay top dollar only for top-quality fiber to be spun into cotton thread.

"The big questions are how many dollars does it cost the farmer to grow a bale of cotton, and what is the fiber in that bale worth to the mill," says Philip A. Miller, ARS national program leader for cotton and oilseed research, headquartered in Beltsville, Maryland. "Bt cotton will be worthwhile if insecticide savings make the difference between those two dollar figures acceptable or more favorable."

Scientists at Monsanto and ARS agree it's too soon to draw firm conclusions about Bt cotton's fiber quality.

In 1990 at the Arizona site, the yield of ginned cotton fiber was the same on the Coker 312 as on the two HD-1 transgenics retested this year. Good news, so far.

But fiber from these two transgenics was coarser than Coker 312—enough to reduce value, says Wilson. On the

DAVID NANCE



Mature cotton boll at left was protected by a gene for Bt, other bolls show damage from cotton pests. (K-4366-8)

other hand, the transgenics at least equaled Coker in fiber length and elasticity, and both had stronger fiber.

"Commercial Bt cotton could be available to farmers by the mid-1990's," says Monsanto senior scientist Randy Deaton. "So far, we're working with five seed companies to backcross Bt strains with their top varieties."

Making those crosses should improve the fiber's qualities, he says. "Most likely, the unusual qualities in some of the Bt-Coker strains are due to the process of tissue-culturing the genetically engineered cells into seed-bearing plants. We don't believe those qualities are due to the Bt gene itself."

Finally, while the case for growing Bt cotton as an alternative to using insecticides appears overwhelming, "we need to do all we can to preserve its effective use in a farmer's field," says Deaton.

That's why Monsanto is developing strategies to keep insects from obtaining resistance to Bt. For example, "we're developing genes that express novel insecticidal proteins and can be

used with Bt," says Deaton. "The insects would find it much more difficult to develop resistance to both genes at the same time."

"We're very proud of this product. But scientists at ARS and the universities will continue to be crucial to getting Bt cotton to the market. Their expertise, accessibility, and openness are making all the difference."—By Jim De Quattro, ARS.

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DAVID NANCE



Plant geneticist David Altman stands among cotton that was not treated for worm pests. In contrast, higher yielding cotton in the background is protected by the Bt gene. (K-4365-13)

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